

REMARKS

Claims 58, 61-66, 70-96, 100-109, 113-116, and 118-121 are pending. Claims 67-69, 97-99, and 117 have been canceled. Applicants reserve the right to file one or more applications directed to the cancelled subject matter.

EXAMINER INTERVIEW

Applicants thank Examiner Quang Nguyen, Ph.D., for the helpful telephonic interview held with the Examiner, Anne Brown, attorney for Applicants, John Harrington, Chief Scientific Officer, Athersys, Inc., and Bruce Sherf, Director of Molecular Biology, Athersys, Inc.

Although the issues discussed in the interview are presented in more detail below, Applicants briefly outline the discussion.

First, Dr. Harrington discussed the rejection of the claims on the basis of new matter. Dr. Harrington referred to a schematic diagram sent to the Examiner for discussion. A copy of that schematic is attached in the document labeled Appendix A.

Dr. Harrington explained that whenever one attempts activation of an endogenous gene by splicing from the vector to an endogenous exon, no protein will be produced from the endogenous gene if the endogenous ATG is in an exon upstream of the exon to which the vector splices. To address this situation, the application discloses including a start codon on the vector exon. By including this codon, if splicing occurs to an endogenous exon that lacks a start codon, a protein will be produced from the endogenous gene. Dr. Harrington pointed out that producing endogenous proteins is one of the fundamental purposes of the invention. He, thus, concluded that a stop codon operably linked to the start codon would defeat the purpose because it would

prevent expression of endogenous protein. Accordingly, he reasoned that such a vector with an ATG implicitly must lack an operably linked stop codon.

The second issue discussed in the interview was the rejection of the claims under 35 U.S.C. § 102(e) over Treco. For this issue, Dr. Sherf explained that the neo gene, first of all, would have to be polyadenylated in order to be stably expressed in a eukaryotic cell. Second, the neo fragment was derived from a commercially available vector containing the neo gene operably linked to a polyA signal. He presented the sequence of the vector that is commercially available and diagrammed where the vector contains the polyadenylation signal operably linked to the neo gene. This information is also in Appendix A.

The third issue that was discussed was the rejection of the claims under 35 U.S.C. § 102(b). Applicants' attorney explained how there was no disclosure in the reference that a vector integrated by *nonhomologous recombination* would cause gene activation. Applicants' attorney provided a citation showing that Treco taught that only homologous recombination events would result in endogenous gene expression. Applicants' attorney further explained how, even if splicing were to occur, the negative marker function is retained. A simple schematic was provided. This is attached in Appendix A. The marker has an independent promoter. Therefore, even if splicing were to occur, the independent transcript would encode a viable expressible negative marker.

Rejection of Claims 58, 61-64, 72-96, 100-109, and 119 Under 35 U.S.C. § 112 First Paragraph, New Matter

On page 2 of the Office Action, claims 58, 61-64, 72-76, 100-109 and 119 have been rejected under 35 U.S.C. § 112, first paragraph, on the grounds that the specification does not reasonably convey the Applicants' possession of the invention. Applicants respectfully traverse the rejection.

The Examiner takes the position that the phrase "contains a translational start codon that is not operably linked to a translational stop codon" is not supported because there is no *literal* support. On page 3 of the Office Action, the Examiner states that the specification discloses a vector construct with an exon containing a translational start codon and unpaired splice donor site. But he also states that there is no literal support that the Applicants would have specifically contemplated the start codon not operably linked to a stop codon.

Regarding the issue of literal support, Applicants point out that black letter patent law holds that support need not be explicit. Implicit support can also reasonably convey to the person of ordinary skill in the art that the Applicant was in possession of the invention. See, for example, *In re Smith and Hubin*, 481 F.2d 910, 178 U.S.P.Q. 620 (C.C.P.A. 1973): compliance with the first paragraph of 35 U.S.C. § 112 is judged from the perspective of the person of ordinary skill in the art. The claimed subject matter need not be described in *haec verba* in the specification in order that the specification satisfy the written description requirement. The specification simply must convey to the person of ordinary skill the art that the Applicant invented the specific claimed subject matter. When the specification accomplishes this, regardless of how it accomplishes it, the essential goal of the description requirement is realized. See also *Ex parte Harvey*, 3 U.S.P.Q.2d 1626 (B.P.A.I. 1986): the test for determining compliance with the written description requirement is whether the disclosure as originally filed reasonably conveys to the artisan that the inventor had possession of the claimed subject matter

rather than the presence or absence of literal support in the specification for the claimed language. Applicants point out that there are many citations supporting this fundamental tenet of patent law.

In the present case, Applicants submit that the claimed embodiment is implicit in Applicants' disclosure. In short, the person of ordinary skill in the art would have envisioned a vector where the vector exon contains a start but not an operably linked stop codon because Applicants' specification teaches the production of endogenous protein where the endogenous start codon is spliced out of the primary transcript. Applicants describe the reasoning in detail below.

A goal discussed throughout the application is protein expression from an endogenous gene by introducing the Applicants' vectors. In some endogenous genes the first exon contains the translation initiation site. If there is no ATG on the vector, then splicing eliminates the translation initiation site on the transcript and protein cannot be produced from the endogenous gene. To address this situation, the specification discloses including a start codon in the vector exon. Then, when splicing occurs, a translatable fusion protein is formed from the vector ATG and endogenous coding sequence.

If a stop codon is operably linked to the start codon on the vector, endogenous protein cannot be produced. If endogenous protein cannot be produced, this defeats the purpose of the start codon. Accordingly, even though there is no explicit verbal recitation that a stop codon is lacking, it would be readily apparent to the person of ordinary skill in the art that in the construction of such a vector, no stop codon is operably linked to the ATG.

The Examiner refers to page 38, construct 3, but states that there is no literal support that such a vector that contains a translational start codon would not be operably linked to a translational stop codon. However, as discussed above, such a structure would not contain a stop codon since this would defeat the purpose of having the start codon in the vector in the first place.

For the Examiner's convenience, Applicants cite the Applicants' specification that support the concepts discussed above.

Page 13, lines 11-15. This text generally discloses the claimed vector but does not describe the design of the activation unit (transcriptional regulatory sequence operably linked to an unpaired splice donor site). There are many choices for constructing this activation unit. One of these choices is to include the start codon in the activation unit, as cited further below.

n another embodiment, the invention provides vector constructs comprising a first transcriptional regulatory sequence operably linked to a selectable marker lacking a polyadenylation signal, and further comprising a second transcriptional regulatory sequences operably linked to an unpaired splice donor site.

Page 16, lines 20-30 to page 17, lines 1-2. The above clearly indicates, as the Examiner has recognized, that the exon can contain a translational start site in a vector designed to produce a polypeptide from the endogenous gene.

The invention also provides additional methods of producing a polypeptide, comprising introducing into a host cell a vector comprising a transcriptional regulatory sequence operably linked to an exonic region followed by an unpaired splice donor site, and culturing the host cell under conditions favoring the expression by said host cell of a polypeptide encoded by the exonic region, wherein the exon contains a translational start site positioned at any of the open reading frame positions relative to the 5'-most base of the unpaired splice donor site (e.g., the "A" in the ATG start codon may be at position -3 or at an increment of 3 bases upstream therefrom (e.g., -6, -9, -12, -15, -18, etc.), at position -2 or at an increment of 3 bases upstream therefrom (e.g., -5, -8, -11, -14, -17, -20, etc.), or at position -1 or at an increment of 3 bases upstream therefrom (e.g., -4, -7, -10, -13, -16, -19, etc.), relative to the 5'-most base of the splice donor site).

Page 35, lines 19-23. This text indicates the purpose discussed above for putting amino acid sequences on the vector, i.e., to produce endogenous protein. The purpose, as discussed above, is to compensate for amino acid sequences in the first exon of the endogenous gene which would be lost when splicing occurs.

Where the sequence of the gene to be activated is known, however, the constructs can be engineered to contain the proper configuration of vector elements (e.g., location of the start codon, addition of codons present in the first exon of the endogenous

gene and the proper reading frame) to achieve maximum overexpression and/or the appropriate protein sequence.

Page 44, lines 15-19 states:

One of the advantages of the methods described herein is that virtually any gene can be activated. However, since genes have different genomic structures, including different intron/exon boundaries and locations of start codons, a variety of activation constructs is provided to activate the maximum number of different genes within a population of cells.

Page 46, lines 25-30 through page 47, lines 1-16. This text also shows the production of endogenous protein when the start codon for the endogenous gene is located in the first exon.

To activate endogenous genes lacking a start codon in their first exon a start codon is preferably omitted from the exon on the vector. To activate endogenous gene containing a start codon in the first exon the exon on the vector preferably contains a start codon, usually ATG and preferably an efficient translation initiation site. The exon may contain additional codons following the start codon. These codons may be derived from a naturally occurring gene or may be non-naturally occurring (e.g., synthetic). The codons may be the same as the codons present in the first exon of the endogenous gene to be activated. Alternative, the codons may be different than the codons present in the first exon of the endogenous gene. ...When the structure of the gene to be activated is known, the splice donor site should be placed adjacent to the vector exon in a location such that the codons in the vector will be in frame with the codons of the second exon of the endogenous gene following splicing. When the structure of the endogenous gene to be activated is not known, separate constructs, each containing a different reading frame, are used.

Page 48, lines 21-27 states:

When the vector is integrated by nonhomologous recombination into a host cell's genome, the unpaired splice donor site becomes paired with a splice acceptor site from an endogenous gene. The splice donor site from the vector, in conjunction with the splice acceptor site from the endogenous gene, will then direct the excision of all of the sequences between the vector splice donor

site and the endogenous splice acceptor site. Excision of these intervening sequences removes sequences that interfere with translation of the endogenous protein.

Page 82, lines 9-24 states:

Alternatively, to increase the frequency of protein expression using non-targeted gene activation, the downstream transcriptional regulatory sequence on the vector may be operably linked to an exonic sequence followed by a splice donor site. In a preferred embodiment, the vector exon lacks a start codon. This vector is particularly useful for activating protein expression from genes that do not encode the translation start codon in exon I. In an alternative preferred embodiment, the vector exon contains a start codon. Additional codons can be located between the translational start codon and the splice donor site. For example, a partial signal secretion sequence can be encoded on the vector exon. The partial signal sequence can be any amino acid sequence capable of complementing a partial signal sequence from an endogenous gene to produce a functional signal sequence. The partial sequence may encode between one and one hundred amino acids, and may be derived from existing genes, or may consist of novel sequences. Thus, this vector is useful for producing and secreting protein from genes that encode part of the endogenous signal sequence in exon I, and the remainder in subsequent exons.

Page 82, lines 29-30 through page 83, lines 1-4 states:

In cases where a start codon is included on the vector exon, it can be advantageous to produce a vector in each reading frame. This is achieved by varying the number of nucleotides between the start codon and the splice donor junction site. Together, the preferred vector configurations are capable of producing protein from endogenous genes, regardless of the exon/intron sequence location of the translation start codon, or reading frame.

All of the above text illustrates how a start codon in the vector is designed to replace the endogenous start codon, lost because of splicing. That being the goal, operably linking a stop codon to the start codon on the vector would destroy the goal by preventing translation of endogenous sequences. Accordingly, Applicants submit that the person of ordinary skill in the

art would have recognized that a stop codon operably linked to the start codon would defeat the purpose of the vector ATG. Therefore, the person of ordinary skill in the art would have readily envisioned the claimed construct. Thus, the Applicants' disclosure meets the test for adequate written description.

In view of the above evidence and discussion, Applicants respectfully submit that the grounds of the rejection have been adequately addressed and the rejection overcome. Reconsideration and withdrawal of the rejection is, therefore, respectfully requested.

Rejection of Claims 58, 61-64, 72-96, 100-109, and 119 Under 35 U.S.C. § 112 First Paragraph, Enablement

On page 3 of the Office Action, claims 58, 61-64, 72-96, 100-109 and 119 have been rejected under 35 U.S.C. § 112, first paragraph, on the grounds that the specification does not enable the claims. Applicants respectfully traverse the rejection.

This rejection is predicated on the propriety of the new matter rejection. As Applicants believe they have overcome that rejection, they also have overcome the non-enablement rejection. Therefore, Applicants request that the Examiner reconsider and withdraw the rejection.

Claims 106 and 107 have been separately rejected on the grounds of non-enablement. The Examiner asserts that the specification does not provide enablement for producing a protein in any cell in which splicing can occur, as set forth in the previous Office Action. Applicants, again, traverse the rejection but suggest that the rejection is moot in view of Applicants' amendment of the claims. Applicants previously argued that even though native prokaryotic cells should not contain splicing enzymes, it would have been possible to transfect prokaryotic cells with splicing enzymes. The Examiner considers the arguments unpersuasive on the grounds that the vectors in the methods as claimed contain only promoters that are functional in a eukaryotic cell and, therefore, the vectors would not be functional in prokaryotic cells.

First, Applicants point out that the claims do not exclude promoters that function in a prokaryotic cell. The claim covers promoters that function in eukaryotes and prokaryotes. What

would be excluded by the claim language would be promoters that do not function in eukaryotic cells, not promoters that function in eukaryotic cells and prokaryotic cells.

This argument notwithstanding, for the sake of expediting prosecution, Applicants have removed the previous amendment and inserted, instead, the proviso that the cell is a eukaryotic cell.

Accordingly, this ground of rejection has been addressed and the rejection overcome. Reconsideration and withdrawal of the rejection on this basis is, therefore, respectfully requested.

Rejection of Claims 58, 61-65, 73, 76, 78-79, 81, 84-87, 89, 92, and 120-121

Under 35 U.S.C. § 102(e)

On page 5 of the Office Action, claims 58, 61-65, 73, 76, 78-79, 81, 84-87, 89, 92 and 120-121 have been rejected under 35 U.S.C. § 102(e) on the grounds that they are anticipated by U.S. Patent Number 6,270,989 (the '989 patent). Applicants respectfully traverse the rejection.

The Examiner cites pRTPO1 (Figure 6). The Examiner states that the neo gene is a bacterial (underline in original) gene "that normally does not contain a polyadenylation signal". Applicants have previously explained why the neo gene and dhfr gene must contain polyadenylation sequences. As Applicants have explained, this marker is used to select a *eukaryotic cell*. The marker cannot stably processed without a polyadenylation signal.

The rejection appears to be based on the fact that Figure 6 in the '989 patent does not explicitly indicate a polyA signal in the neo gene. However, it is standard in schematic vector diagrams to show the gene only and not the associated transcriptional and translation regulatory sequences. Accordingly, there is an initiation codon, a cap site, a promoter, etc., but these elements are not shown in Figure 6 either.

In the interview held with the Examiner on February 27, 2004, Dr. Bruce Sherf, Director of Molecular Biology, Athersys, Inc., explained that the origin of the neo gene in plasmid pRTPO1 is a commercially available vector that can be obtained from Stratagene, Inc. As described in U.S. 6,270,989 B1 (column 20, line 38), the neomycin gene of plasmid "pRTPO1" is derived from the 1.15 KB Xho1/Sal1 DNA fragment of commercial plasmid "pMC1neoPolyA" (Stratagene, Inc.). The circle map of pMC1neoPolyA (see Appendix A, attached) is printed directly from the Stratagene website

(<http://www.stratagene.com/lit/vector.aspx>) and depicts the Xho1 to Sal1 cassette to include the TK promoter, neomycin open reading frame, and poly-adenylation region. The full DNA sequence of pMC1neoPolyA is available from the Stratagene website, as well as through Genbank (accession # U43612). The enclosed (Appendix A) sequence of pMC1neoPolyA is annotated by Dr. Sherf to clarify the positions of the TK promoter, neomycin ORF, and poly-A regions relative to the Xho1 and Sal1 subcloning sites. The polyA sequence used in pMC1neoPolyA and pRTPO1 vectors is commonly used in the construction of gene expression vectors; it is derived from the HSV TK gene (for reference, see Genbank accession #X03896).

As discussed in the interview, the other marker, an amplifiable marker, dhfr, is a eukaryotic mammalian gene. The Examiner has not taken the position that this gene would not contain a polyadenylation signal. Accordingly, pRTPO1 does not meet the limitations in the claim.

In view of the above discussion, Applicants respectfully submit that the grounds for rejection have been addressed and the rejection overcome. Reconsideration and withdrawal of the rejection is, therefore, requested.

Rejection of Claims 70, 73-79, 81-87, 89-92, and 113-116 Under 35 U.S.C. § 102(b)

On page 7 of the Office Action, claims 70, 73-79, 81-87, 89-92 and 113-116 have been rejected under 35 U.S.C. § 102(b) on the grounds that they are anticipated by WO 95/31580. Applicants respectfully traverse the rejection.

First, Applicants point out that the same vector construct (Treco's positive/negative marker construct) was cited against the following issued claim in Applicants' U.S. Patent Number 6,623,958. Yet the Patent Office allowed the claim.

1. A vector comprising:
 - (a) a first promoter;
 - (b) a sequence encoding a positive selectable marker
 - (c) a sequence encoding a negative selectable marker; and
 - (d) an unpaired splice donor site,wherein said sequence encoding said positive selectable marker, said sequence encoding said negative selectable marker and said splice donor site are oriented in said vector in an orientation that, when said vector is integrated into the genome of a eukaryotic host cell in such a way that splicing occurs between said splice donor

site and a splice acceptor site in the host cell genome, then said positive selectable marker is expressed in active form and said negative selectable marker is either not expressed or is expressed in inactive form.

On page 9 of the Office Action, the Examiner states “it should be noted that the negatively selectable marker genes of the DNA constructs of Treco et al, which have been incorporated randomly into genomes of transfected cells, would be deleted through a splicing event due to the presence of the unpaired splice donor”. First, Applicants respectfully submit that there is no indication from the Treco reference that nonhomologous recombination events are productive. In fact, the reference specifically leads the reader to believe that no activation events will occur unless the vector is homologously recombined. This was discussed in the interview.

On page 43, the reference states that most of the integration events that occur are nonhomologous or illegitimate recombination events. However, in discussing activation of the EPO gene on page 71 of the reference, the reference states that cells in which the transfecting DNA integrates randomly into the human genome cannot produce EPO. Accordingly, Treco does not disclose the recovery of cells in which gene activation follows nonhomologous recombination events and, in fact, discloses that only homologously recombinant cells express the desired gene.

It was, in fact, the Applicants who determined that nonhomologous integration events could activate endogenous genes.

More to the point, and discussed in detail in the interview, even if splicing were to occur, the Treco vector would not meet the limitations of the claims. Applicants refer to the schematic under the heading “102(b)” in the attached Appendix A. Even if gene activation and splicing were to occur with the non-homologously recombined vectors, this still would not eliminate expression of the negatively selectable marker. The negatively selectable marker is transcribed from its own independent promoter, so an independent transcript is produced that encodes the negatively selectable marker. Splicing would have no effect on this transcript. Thus, the cited vector is not designed so that, upon integration and splicing, the negatively selectable marker is mis-expressed.

Applicants point out that the Examiner recognizes that the negatively selectable marker contains a promoter. See the first sentence of the paragraph spanning pages 8 and 9. Thus, the

Examiner will recognize that two independent transcripts are produced that encode the selectable marker.

In view of the above discussion, Applicants submit that all grounds of rejection have been addressed and the rejection overcome. Reconsideration and withdrawal of the rejection is, therefore, requested.

Rejection of Claims 67-69, 74-75, 77, 79, 82-83, 85-87, 90-91, 94-96, 97-101, and 119

Under 35 U.S.C. § 102(e)

On page of the Office Action, claims 67-69, 74-75, 77, 79, 82-83, 85-87, 90-91, 94-96, 97-101 and 119 are rejected on the grounds that they are anticipated by U.S. Patent Number 6,139,833. Applicants respectfully traverse the rejection.

The rejection is moot since Applicants have cancelled the claim.

Applicants submit that the grounds for rejection have been addressed and the rejection overcome. Reconsideration and withdrawal of the rejection is, therefore, requested.

Application No.: 09/484895
Examiner: Q. Nguyen

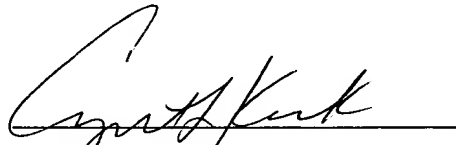
Docket No.: ATX-007CP4DV7
Art Unit: 1636

SUMMARY

In view of the remarks set forth above, it is respectfully submitted that this application is in condition for allowance. If there are any remaining issues or the Examiner believes that a telephone conversation with Applicants' attorney would expedite the prosecution of the above-identified application, the examiner is urged to call Applicant's attorney at (617) 227-7400.

Respectfully submitted,

LAHIVE & COCKFIELD, LLP

A handwritten signature in dark ink, appearing to read 'Cynthia L. Kanik', written over a horizontal line.

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For

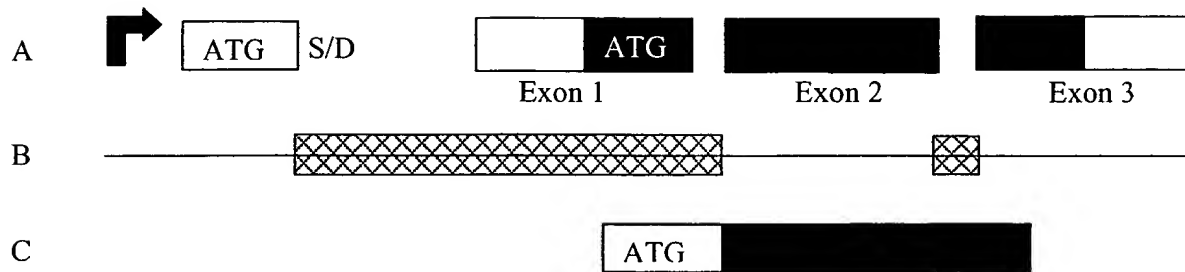
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Dated: March 8, 2004

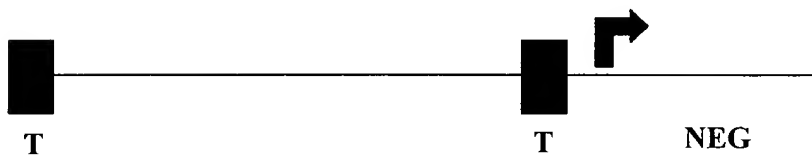


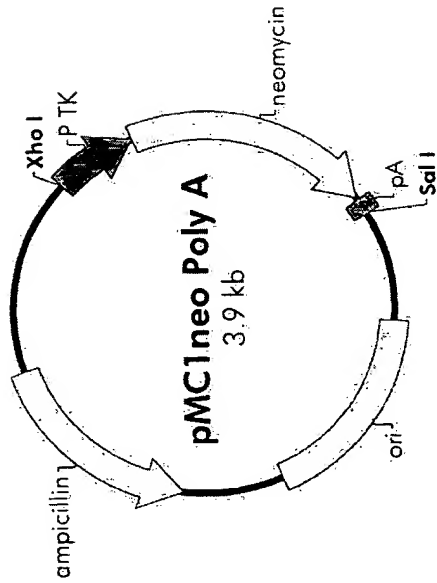
Appendix A



- A Vector and endogenous gene
Shaded area is translated region in gene
- B Primary transcript; hatched boxes show spliced out transcript
- C Final fusion protein with vector ATG replacing exon 1 ATG

102(b)





Settings : Circular, Certain Sites Only, Standard Genetic Code

CTGCCTCGCGCTTTTCGGTGATGACGGTGAAAACCTCTGACACATGACAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGG
90
GACGGAGCGCGCAAAGCCACTACTGCCACTTTTGGAGACTGTGTACGTCGAGGGCCTCTGCCAGTGTGAAACAGACATTCCGCTACGGCC
GAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTGGCGGGTGTGGGGCGCAGCCATGACCCAGTCACGTAGCGATAGCGGAGTGTA
180
CTCGTCTGTTTCGGGAGTCCCGCGCAGTCGCCACAACCGCCACAGCCCCGCGTCGGTACTGGGTAGTGCATCGCTATCGCCTCACAT
CTGGCTTAACATATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAATA
270
GACCGAATTGATACGCCGTAGTCTCGTCTAACATGACTCTCACGTGGTATACGCCACACTTTATGGCGTGTCTACGCATTCTCTTTTAT
CCGCATCAGGGGCCATTGCCATTACGGCTACGCAACTGTTGGGAAGGGCGATCGGTGCGGGCTCTTCGCTATTACGCCAGCTGGCGAA
360
GGCGTAGTCCGCGTAAGCGGTAAGTCCGATGCGTTGACAACCTTCCCGCTAGCCACGCCCGGAGAAGCGATAATGCGGTGACCGCTT
GGGGGATGTGCTGCAAGGCGATTAAAGTTGGGTAACGCCAGGGTTTTCCAGTCACGACGTTGTAAACGACGCCAGGGCCAGTGAATT
450
CCCCCTACACGACGTTCCGCTAATTCAACCCATTGCGGTCCCAAAGGGTCAGTGTGCAACATTTTGCTGCCGGTCCCGGTCACTTAA
XhoI *Subcloning*
Site
CTCGAGCAGTGTGGTTTTGCAAGAGGAAGCAAAAAGCCTCTCCACCCAGGCCTGGAATGTTTCCACCCAATGTCGAGCAGTGTGGTTTTG
540
GAGCTCGTCACACCAAAACGTTCTCTTCGTTTTTCGGAGAGGTGGGTCCGGACCTTACAAAGGTGGGTTACAGCTCGTCACACCAAAAC
TK Promoter
CAAGAGGAAGCAAAAAGCCTCTCCACCCAGGCCTGGAATGTTTCCACCCAATGTCGAGCAAAACCCGCCAGCGCTTGTGCTATTGGCGAA
630
GTTCTCCTTCGTTTTTCGGAGAGGTGGGTCCGGACCTTACAAAGGTGGGTTACAGCTCGTTTGGGGCGGGTCGAGAACAGTAACCGCTT
TK Promoter
TTCGAACACGCAGATGCAGTCGGGGCGGCGGGTCCAGGTCCACTTCGCATATTAAGGTGACGCGTGTGGCTCGAACACCGAGCGACC
720
AAGCTTGTGCGTCTACGTCAGCCCCGCGCGCCAGGGTCCAGGTGAAGCGTATAATTCCACTGCGCACACCGAGCTTGTGGCTCGCTGG
TK Promoter
CTGCAGCCAATATGGGATCGGCCATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCCGCTATGACT
810
GACGTCGGTTATACCTAGCCGTAACTTGTTCTACCTAACGTGCGTCCAAGAGCGCGGCAACCCACCTCTCCGATAAGCCGATACTGA
M G S A I E Q D G L H A G S P A A W V E R L F G Y D
Neomycin
GGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTGACGCGAGGGGCGCCCGGTTCTTTTGTCAAGACCGACCTGT
900
CCCGTGTGCTGTTAGCCGACGAGACTACGGCGGCACAAGGCCGACAGTCGCGTCCCGCGGGCCAAGAAAAACAGTTCTGGCTGGACA
W A Q Q T I G C S D A A V F R L S A Q G R P V L F V K T D L
Neomycin
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990
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S G A L N E L Q D E A A R L S W L A T T G V P C A A V L D V
Neomycin
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1080
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V T E A G R D W L L L G E V P G Q D L L S S H L A P A E K V
Neomycin



CCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAGCGAG 1170
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S I M A D A M R R L H T L D P A T C P F D H Q A K H R I E R
Neomycin

CACGTA CTGGATGGAAGCCGGTCTTGTGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCCGCGAGGC 1260
GTGCATGAGCTACCTTCGCCAGAACAGCTAGTCCTACTAGACCTGCTTCTCGTAGTCCCCGAGCGCGGTTCGGCTTGACAAGCGGTCCG
A R T R M E A G L V D Q D D D E E H Q G L A P A E L F A R
Neomycin

TCAAGGCGCGCATGCCGACGGCGAGGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTT 1350
AGTTCGCGCGGTACGGGCTGCCGCTCCTAGAGCAGCACTGGGTACCGTACGGACGAACGGCTTATAGTACCACCTTTTACCGCGCAAAA
L K A R M P D G E D L V V T H G D A C L P N I M V E N G R F
Neomycin

CTGGATTTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCG 1440
GACCTAAGTAGCTGACACCGGCCGACCCACACCGCTGGCGATAGTCTGTATCGCAACCGATGGGCACTATAACGACTTCTCGAACCGC
S G F I D C G R L G V A D R Y Q D I A L A T R D I A E E L G
Neomycin

GCGAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTGCGAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCT 1530
CGCTTACCCGACTGGCGAAGGAGCACGAAATGCCATAGCGCGGAGGGCTAAGCGTCCGCTAGCGGAAGATAGCGGAAGAACTGCTCAAGA
G E W A D R F L V L Y G I A A P D S Q R I A F Y R L L D E F
Neomycin

ref. GenBank X03896

BamHI (Sal I) Subcloning Site

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F
- Neomy

HSV TK poly A

TAATCATGGTCATAGCTGTTTCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAGCC 1710
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TAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCCTATTGGCGCTCTTCGCTTCTCGCTCACTGACTCGCTCGGTCGGTCGTTT 1890
ATTACTTAGCCGGTTGCGCGCCCTCTCCGCCAACGCATAACCGCGAGAAGCGCAAGGAGCGAGTGACTGAGCGACGCGAGCCAGCAAG

GGCTGCGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAA 1980
CCGACGCCGCTCGCCATAGTCAGTGAGTTTCCGCCATTATGCCAATAGGTGCTTAGTCCCTATTGCGTCCTTTCTTGTAACACTCGTT

AAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCGCGTTTGTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATC 2070
TTCCGGTCGTTTTCCGGTCCTTGGCATTTTTCCGGCGCAACGACCGCAAAAAGGTATCCGAGGCGGGGGGACTGCTCGTAGTGTTTTTAG

GACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACGAGCGGTTTCCCTTGAAGCTCCCTCGTGGCTCTCTGTGTC 2160
CTGCGAGTTCAGTCTCCACCGCTTTGGGCTGTCTGATATTTCTATGGTCCGCAAGGGGGACCTTCGAGGGAGCACGCGAGAGGACAAG

CGACCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAATGCTCAGCTGTAGGTATCTCAGTT 2250
GCTGGGACGGCGAATGGCTATGGACAGGCGGAAAGAGGGAAGCCCTTCGCACCGGAAAGAGTTACGAGTGCACATCCATAGAGTCAA

CGGTGTAGGTGCTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCGTTACGCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTG 2340
GCCACATCCAGCAAGCGAGGTTGACCCGACACAGTGTCTGGGGGGCAAGTCGGGCTGCGACGCGGAATAGGCCATTGATAGCAGAAC

AGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAG 2430
TCAGGTTGGGCCATTCTGTGCTGAATAGCGGTGACCGTCTGCGGTGACCATTTGTCTTAATCGTCTCGCTCCATACATCCGCCACGATGTC

AGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAA 2520
TCAAGAACTTCACCACCGGATTGATGCCGATGTGATCTTCTGTCTATAAACCATAGACGCGAGACGACTTCGGTCAATGGAAGCCTTTTT

GAGTTGGTAGCTCTTGATCCGGCAAACAACACCGCTGGTAGCGGTGGTTTTTTTGTITGCAAGCAGCAGATTACGCGCAGAAAAAAG 2610
CTCAACCATCGAGAACTAGGCCGTTTGTITGGTGGCGACCATCGCCACCAAAAAACAACGTTCTGTCGTCTAATGCGCGTCTTTTTTTC

GATCTCAAGAAGATCCTTTGATCTTTTCTACGGGCTGACGCTCAGTGAACGAAAACCTACGTTAAGGGATTTTGGTCATGAGATTAT 2700
CTAGAGTTCTTCTAGGAACTAGAAAAGATGCCCCAGACTGCGAGTCACCTTGCTTTTGAGTGCAATTCCCTAAAACAGTACTCTAATA

CAAAAAGGATCTTCACCTAGATCCTTTTAAATAAAAATGAAGTTTTAAATCAATCTAAAGTATATAGAGTAACTTGGTCTGACAGTT 2790
GTTTTCTCTAGAAGTGGATCTAGGAAAATTAATTTTACTTCAAATTTAGTTAGATTTATATATACTCATTGAACCAGACTGTCAA

ACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTTCGTTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTA 2880
TGGTTACGAATTAGTCACTCCGTGGATAGAGTCGCTAGACAGATAAAGCAAGTAGGTATCAACGGACTGAGGGGCAGCACATCTATTGAT

CGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACC 2970
GCTATGCCCTCCCGAATGGTAGACCGGGGTACGACGTTACTATGGCGCTCTGGGTGCGAGTGGCCGAGGTCTAAATAGTCGTTATTGG

AGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCTGCAACTTTATCCGCTCCATCCAGTCTATTAATTGTTGCCGGAAGCTAGAGTAA 3060
TCGGTGGCCTTCCCGGCTCGCGTCTCACCAGGACGTTGAAATAGCGGAGGTAGGTGAGATAATTAACAACGGCCCTTCGATCTCATT

GTAGTTCGCCAGTTAATAGTTTGCACAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCGTTTGGTATGGCTTCATTCA 3150
CATCAAGCGGTCAATTATCAAACGCGTTGCAACAACGGTAACGATGTCGCTAGCACCACAGTGCAGCAGCAAACCATACCGAAGTAAGT

GCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCCTCCGATCGTTGTCA 3240
CGAGGCCAAGGGTTGCTAGTTCCGCTCAATGTACTAGGGGGTACAACACGTTTTTTCGCCAATCGAGGAAGCCAGGAGGCTAGCAACAGT

GAAGTAAGTTGGCCGAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCTATGCCATCCGTAAGATGCTTTTCTG 3330
CTTCATTCAACCGGCTCACAATAGTGAGTACCAATACCGTCGTGACGTATTAAGAGAATGACAGTACGGTAGGCATTCTACGAAAAGAC

TGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCG 3420
ACTGACCACTCATGAGTTGGTTCAAGTACTTATCACATACGCCGCTGGCTCAACGAGAACGGGCCGAGTTATGCCCTATTATGGC

CGCCACATAGCAGAACTTTAAAAGTGCTCATCATTTGAAAACGTTCTTCGGGGCGAAAACCTCAAGGATCTTACCGCTGTTGAGATCCA 3510
GCGGTGTATCGTCTTGAAATTTTACGAGTAGTAACCTTTTGCAAGAAGCCCCGCTTTTGAGAGTTCTTAGAATGGCGACAACCTTAGGT

GTTGATGTAACCCACTCGTGCACCCAAC TGATCTTCAGCATCTTTTACTTTTACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAA 3600
CAAGCTACATTGGGTGAGCACGTGGGTGACTAGAAAGTCGTAGAAAATGAAAGTGGTCGCAAAGACCCACTCGTTTTTGTCTTCCGTTT

ATGCCGCAAAAAGGGAATAAGGGCGACACGAAATGTTGAATACTCATACTCTTCCTTTTCAATATTATTGAAGCATTATCAGGGTT 3690
TACGGCGTTTTTCCCTTATCCCGCTGTGCCTTTACAACCTATGAGTATGAGAAGGAAAAAGTTATAATACTTCGTAAATAGTCCCAA

ATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAACAAATAGGGGTCCGCGCACATTTCCCGGAAAAGTGCCACCTG 3780
TAACAGAGTACTCGCCTATGTATAAACTTACATAAATCTTTTTATTGTTTATCCCAAGGCGCGTGTAAGGGGCTTTTCACGGTGGAC

ACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAAATAGGCGTATCAGAGGCCCTTTTCGTCTTCAAGAA 3857
TGCAGATTCTTTGGTAATAATAGTACTGTAATTGGATATTTTATCCGCATAGTGCTCCGGGAAAGCAGAAGTTCTT

As described in US 6,270,989 B1 (column 20, line 38, the neomycin gene of plasmid "pRTPO1" (Fig. 6 of) is derived from the 1.15 Kb Xho1/Sal1 DNA fragment of commercial plasmid "pMC1neoPolyA" (Stratagene, Inc). The enclosed circle map of pMC1neoPolyA is printed directly from the Stratagene website (<http://www.stratagene.com/lit/vector.aspx>) and depicts the Xho1 to Sal1 cassette to include the TK promoter, neomycin open reading frame, and poly-adenylation region. The full DNA sequence of pMC1neoPolyA is available from the Stratagene website, as well as through Genbank (accession # U43612). The enclosed sequence of pMC1neoPolyA is annotated by us to clarify the positions of the TK promoter, neomycin ORF, and poly-A regions relative to the Xho1 and Sal1 subcloning sites. The poly A sequence used in pMC1neoPolyA and pRTPO1 vectors is commonly used in the construction of gene expression vectors; it is derived from the HSV TK gene (for reference, see Genbank accession #X03896).